PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

# PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

| | |
|---|---|
| **Date of mailing** (day/month/year)<br>04 May 2000 (04.05.00) | |
| **International application No.**<br>PCT/CA99/00844 | **Applicant's or agent's file reference**<br>12667-16PCT |
| **International filing date** (day/month/year)<br>15 September 1999 (15.09.99) | **Priority date** (day/month/year)<br>17 September 1998 (17.09.98) |
| **Applicant**<br>CHEMTOB, Sylvain et al | |

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

10 April 2000 (10.04.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

| | |
|---|---|
| **The International Bureau of WIPO**<br>**34, chemin des Colombettes**<br>**1211 Geneva 20, Switzerland** | Authorized officer<br>Olivia RANAIVOJAONA |
| Facsimile No.: (41-22) 740.14.35 | Telephone No.: (41-22) 338.83.38 |

m·H

**PCT** WORLD INTELLECTUAL PROPERTY ORGANIZ/
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/12, C07K 14/705, 14/72, A61K 38/04, A61P 15/06, G01N 33/50, 33/68

A1

(11) International Publication Number: **WO 00/17348**

(43) International Publication Date: 30 March 2000 (30.03.00)

(21) International Application Number: PCT/CA99/00844

(22) International Filing Date: 15 September 1999 (15.09.99)

(30) Priority Data:
09/154,627 17 September 1998 (17.09.98) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 09/154,627 (CIP)
Filed on 17 September 1998 (17.09.98)

(71) Applicant *(for all designated States except US)*: HOPITAL SAINTE-JUSTINE [CA/CA]; 3175 Côte Sainte-Catherine, Montréal, Québec H3T 1C5 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: CHEMTOB, Sylvain [CA/CA]; 6885 Banting, Montréal, Québec H4W 1G1 (CA). PERI, Krishna, G. [CA/CA]; #315, 3555 Atwater Avenue, Montréal, Québec H3H 1Y3 (CA).

(74) Agents: CÔTÉ, France et al.; Swabey Ogilvy Renault, Suite 1600, 1981 McGill College Avenue, Montréal, Québec H3A 2Y3 (CA).

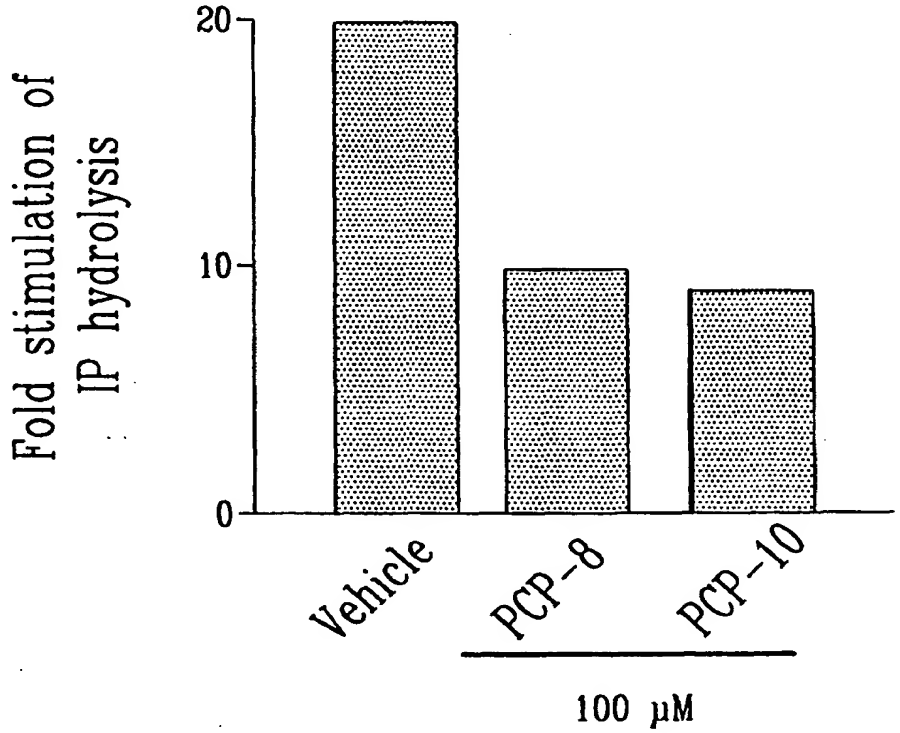
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published**
*With international search report.*
*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: G PROTEIN-COUPLED RECEPTOR AGONISTS OR ANTAGONISTS

(57) Abstract

The present invention relates to a new class of G protein-coupled receptor agonist or antagonist, which specifically binds to the receptor protein structural elements, thus altering signal transmission and subsequent physiological effects. Described herein are peptide sequences derived from the G protein-coupled receptor protein, produced by chemical methods as selective inhibitors of signal transduction associated with stimulation of the receptor by its ligand. Such peptides or molecules derived from their primary, secondary or tertiary structures may be used as effective tocolytics for the prevention of premature labor or be used for the treatment of dysmenorrhea.



*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AL** | Albania | **ES** | Spain | **LS** | Lesotho | **SI** | Slovenia |
| **AM** | Armenia | **FI** | Finland | **LT** | Lithuania | **SK** | Slovakia |
| **AT** | Austria | **FR** | France | **LU** | Luxembourg | **SN** | Senegal |
| **AU** | Australia | **GA** | Gabon | **LV** | Latvia | **SZ** | Swaziland |
| **AZ** | Azerbaijan | **GB** | United Kingdom | **MC** | Monaco | **TD** | Chad |
| **BA** | Bosnia and Herzegovina | **GE** | Georgia | **MD** | Republic of Moldova | **TG** | Togo |
| **BB** | Barbados | **GH** | Ghana | **MG** | Madagascar | **TJ** | Tajikistan |
| **BE** | Belgium | **GN** | Guinea | **MK** | The former Yugoslav Republic of Macedonia | **TM** | Turkmenistan |
| **BF** | Burkina Faso | **GR** | Greece | | | **TR** | Turkey |
| **BG** | Bulgaria | **HU** | Hungary | **ML** | Mali | **TT** | Trinidad and Tobago |
| **BJ** | Benin | **IE** | Ireland | **MN** | Mongolia | **UA** | Ukraine |
| **BR** | Brazil | **IL** | Israel | **MR** | Mauritania | **UG** | Uganda |
| **BY** | Belarus | **IS** | Iceland | **MW** | Malawi | **US** | United States of America |
| **CA** | Canada | **IT** | Italy | **MX** | Mexico | **UZ** | Uzbekistan |
| **CF** | Central African Republic | **JP** | Japan | **NE** | Niger | **VN** | Viet Nam |
| **CG** | Congo | **KE** | Kenya | **NL** | Netherlands | **YU** | Yugoslavia |
| **CH** | Switzerland | **KG** | Kyrgyzstan | **NO** | Norway | **ZW** | Zimbabwe |
| **CI** | Côte d'Ivoire | **KP** | Democratic People's Republic of Korea | **NZ** | New Zealand | | |
| **CM** | Cameroon | | | **PL** | Poland | | |
| **CN** | China | **KR** | Republic of Korea | **PT** | Portugal | | |
| **CU** | Cuba | **KZ** | Kazakstan | **RO** | Romania | | |
| **CZ** | Czech Republic | **LC** | Saint Lucia | **RU** | Russian Federation | | |
| **DE** | Germany | **LI** | Liechtenstein | **SD** | Sudan | | |
| **DK** | Denmark | **LK** | Sri Lanka | **SE** | Sweden | | |
| **EE** | Estonia | **LR** | Liberia | **SG** | Singapore | | |

# G PROTEIN-COUPLED RECEPTOR AGONISTS OR ANTAGONISTS

## BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to development of agonist or antagonist of a G protein-coupled receptor, which bind to the G protein-coupled receptor from the extracellular side in a manner different from that of the natural ligand.

(b) Description of Prior Art

Prostaglandins are derived from the oxygenation of arachidonic acid by prostaglandin synthetases. Prostaglandins mediate a wide variety of physiological actions, such as vasomotricity, sleep/wake cycle, intestinal secretion, lipolysis, glomelular filtration, mast cell degranulation, neurotransmission, platelet aggregation, leuteolysis, myometrial contraction and labor, inflammation and arthritis, patent ductus arteriosus, cell growth and differentiation (Coleman, R.A. et al., 1994, *Pharmacol. Rev.* **46**:205-229; Goetzl, E.J. et al., 1995, *FASEB J.* **9**:1051-10585). Prostanoids mediate their actions through binding to distinct receptors, which belong to the super family of rhodopsin-like seven transmembrane helical receptors. These receptors are coupled to heterotrimeric G-proteins comprising of $\alpha$, $\beta$ and $\gamma$ subunits which, upon activation, elicit alterations in cell calcium, initiate phosphoinositide hydrolysis or promotion or repression of cyclic adenosine monophosphate synthesis (Strader C. D. et al., 1994, *Ann. Rev. Biochem.* **63**:101-132).

Of the five pharmacologically distinct prostanoid receptors for $PGE_2$, $PGI_2$, $PGD_2$, $PGF_{2\alpha}$ and $TxA_2$ and their many isoforms, the receptor for $PGF_{2\alpha}$, also called FP receptor, shows limited tissue distribution, predominantly expressed in corpora leutea, uterine myome-

trium, trabecular meshwork of the eye, and to a lesser extent in vascular smooth muscle. Initiation of labor is marked by tremendous rise in $PGF_{2\alpha}$ levels and increased uterine contractility. The wide spread use of $PGF_{2\alpha}$ analogues to induce labor in veterinary industry points to the primary role of $PGF_{2\alpha}$ and its receptor in parturition. This is underscored by the fact that mice lacking the FP receptor fail to undergo labor (Sugimoto et al., 1997, *Science* **277**:81-83). In face of escalating costs incurred as a result of premature births and associated complications to the neonate, such as intraventricular hemorrhage, bronchopulmonary displasia and periventricular leukomalacia leading to cerebral palsy, prolongation of gestation by arresting premature labor is an effective preventive therapy. The relative success of nonsteroidal anti-inflammatory drugs as a short-term therapy toward prevention of premature labor is based on their inhibitory actions upon the synthesis of prostaglandins, particularly $PGE_2$ and $PGF_{2\alpha}$. However, inhibition of $PGE_2$ is associated with serious complications to the fetus such as the closure of ductus arteriosus, renal failure and pulmonary hypertension.

At another level, $PGF_{2\alpha}$ has been attributed a major role in dysmenorrhea, a condition which afflicts 5%-7% of premenopausal women. A pre-menstrual increase in $PGF_{2\alpha}$ levels resulting in myometrial spasms underlies the pathogenesis of this disorder. Lack of effective antagonists of FP receptor for extended therapy hampered the advances in preventing premature labor and associated sequelae, and the design of such antagonists is the subject of this application.

Human FP receptor is a 45 kDa integral membrane glycoprotein, consisting of 359 amino acids and shares only 47% sequence identity with $EP_1$ receptor, and to a lesser extent with other prostanoid receptors

(Abramovitz et al., 1994, *J. Biol. Chem.* **269**:2632-2636). Binding of $PGF_{2\alpha}$ to FP receptor is followed by the activation of $G_{\alpha q\beta\gamma}$ complex, increased GTP binding by the $G_{\alpha q}$ subunit, stimulation of phospholipase Cβ activity, release of inositol phosphates, increased intracellular calcium and subsequent signal transduction phenomena ultimately leading to smooth muscle contraction (Coleman, R.A. et al., 1994, *Pharmacol. Rev.* **46**:205-229). The FP receptor is the only efficacious target for development of therapeutic drugs since a few $G_{\alpha}$-proteins catalyze the actions of hundreds of G-protein coupled receptors, thus targets downstream from the receptor are essentially of little use.

Antagonists of FP receptors directed to the ligand binding site could be of limited use since ligand based inhibitors show cross reactivity with other prostanoid receptors. Their efficacy will be compromised in face of tremendous increase in $PGF_{2\alpha}$ concentrations in myometrium at the onset of labor and in menstruation. The high basal activity of the receptors in the absence of ligand limits the use of ligand-based inhibitors.

It would be highly desirable to be provided with agonist or antagonist of FP receptors, which do not crossreact with other prostanoid receptors, and are effective even in the presence of excess ligand.

## SUMMARY OF THE INVENTION

One aim of the present invention is to provide agonist or antagonist of FP receptors, which do not crossreact with other prostanoid receptors.

Another aim of the present invention is to provide activators or inhibitors of FP receptors by a novel strategy to target the extracellular domains of the receptor protein.

In accordance with the present invention, there is provided a G protein-coupled receptor agonist or antagonist which specifically binds to the juxtamembrane extracellular structural elements of the G protein-coupled receptor in a manner different from that of the natural ligand, and wherein said agonist or antagonist alter the transduction of an intracellular signal. The G protein-coupled receptor agonist or antagonist may be derived from the amino acid sequence of the receptor.

In accordance with a preferred embodiment of the present invention, the agonist or antagonist does not crossreact with other prostanoid receptors.

The antagonist is effective in the presence of excess ligand.

The agonist or antagonist may preferably comprise an amino acid sequence derived from the first and/or second extracellular loops of prostanoid receptors.

In accordance with another embodiment of the present invention, the antagonists of the present invention comprise amino acid sequences derived from the first and second extracellular loops of prostanoid receptors.

In accordance with a preferred embodiment of the present invention, the G protein-coupled receptor is the prostaglandin $F_{2\alpha}$ receptor (FP receptor).

In accordance with a preferred embodiment of the present invention, the antagonist of the present invention comprises amino acid sequences derived from the prostaglandin $F_{2\alpha}$ receptor.

Preferably, the antagonist include, without limitation, amino acid sequence of the FP receptor selected from the group consisting of ILGHRDYK (PCP-8; SEQ ID NO:1); WEDRFYLL (PCP-10; SEQ ID NO:2); YQDRFYLL

(PCP-14; SEQ ID NO:3); ILAHRDYK (PCP-13.7; SEQ ID NO:4); ILGFRDYK (PCP-13.11; SEQ ID NO:5); ILGHKDYK (PCP-13.13; SEQ ID NO:6); ILGHRNYK (PCP-13.14; SEQ ID NO:7); ILGHQDYK (PCP-13.18; SEQ ID NO:8); ILGHRDY-amide (PCP-13.20; SEQ ID NO:9); ILGHRDYK-amide (PCP-13.21; SEQ ID NO:1); ILGWRDYK (PCP-13.22; SEQ ID NO:10); ILGXRDYK (PCP-13.24; SEQ ID NO:11); SNVLCSIF (PCP-15; SEQ ID NO:12) protein fusions and peptidomimetics thereof; wherein said amino acid sequence contains L- and/or D-amino acid.

In accordance with the present invention, there is provided a peptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1 to 12 and wherein said amino acid sequence contains L- and/or D-amino acid, an amino acid sequence with at least about 90% homology to SEQ ID NO:1 to 12, and peptidomimetic thereof.

In accordance with the present invention, there is provided a pharmaceutical composition containing at least a G protein-coupled receptor agonist and antagonist of the present invention, mixture thereof, or functional derivatives thereof in association with a pharmaceutically acceptable carrier.

In accordance with another embodiment of the present invention, there is provided a method for preventing premature delivery of fetus, which comprises the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist or functional derivatives thereof, wherein the antagonist or functional derivatives thereof specifically binds to the extracellular face of the receptor, thereby hampering uterine contractions.

In accordance with another embodiment of the present invention, there is provided a method for pre-

venting and/or treating dysmenorrhea comprising the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist or functional derivatives thereof, wherein the antagonist or functional derivatives thereof specifically binds to the extracellular face of the receptor to hamper transduction of a signal thereby reducing the pain associated with contractions.

In accordance with another embodiment of the present invention, there is provided a method of identifying a compound as a G protein-coupled receptor agonist or antagonist capable of binding to the extracellular elements of the said receptor in a manner different from that of the natural ligand, comprising the steps of:

a) culturing cells which express said receptor or identifying animal tissues *ex vivo* or *in vivo* where physiological consequences are dependent on said receptor;

b) contacting said cells or tissues with said compound to be tested for agonist or antagonist activity at said receptor; and

c) measuring a response to alter the transduction of a signal resulting in physiological consequences selected from the group consisting of increments in cell calcium, phosphoinositide hydrolysis, increased/decreased cellular cyclic adenosine monophosphate, cell growth and/or differentiation, altered gene expression, and smooth muscle contraction or dilation, wherein said response is indicative of agonist or antagonist activity.

In accordance with another embodiment of the present invention, there is provided a method of iden-

tifying a compound as a prostaglandin $F_2$ alpha receptor agonist or antagonist capable of binding to the extracellular elements of the said receptor in a manner different from that of the natural ligand, comprising the steps of:

a) culturing cells which express said receptor or identifying animal tissues *ex vivo* or *in vivo* where physiological consequences are dependent on said receptor;

b) contacting said cells or tissues with said compound to be tested for agonist or antagonist activity at said receptor; and

c) measuring a response to alter the transduction of a signal resulting in physiological consequences selected from the group consisting of increments in cell calcium, phosphoinositide hydrolysis, cell growth and/or differentiation, altered gene expression, and smooth muscle contraction or dilation, wherein said response is indicative of agonist or antagonist activity.

For the purpose of the present invention the following terms are defined below.

The expression "a G protein-coupled receptor agonist or antagonist" is intended to mean any natural or synthetic compound, peptide protein, antibody, peptidomimetic or small chemical molecules, without limitation, insofar as it can specifically bind to the extracellular structural elements of the G protein-coupled receptor to alter transduction of a signal. More preferably, the agonist or antagonist does not crossreact with other prostanoid receptors.

The expression "functional derivatives" of G protein-coupled receptor agonist or antagonist is intended to mean mimetic compounds and/or structurally unrelated compounds with respect to G protein-coupled

receptor antagonist, which can also specifically bind to the extracellular structural elements of the G protein-coupled receptor to alter transduction of a signal.

The expression "peptidomimetic thereof" is intended to mean any chemical entities, mimetic compounds and/or structurally unrelated compounds with respect to G protein-coupled receptor agonist or antagonist, which can also specifically bind to the extracellular structural elements of the G protein-coupled receptor to alter transduction of a signal.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Fig. 1 illustrates the inhibitory effects of PCP-8 and PCP-10 on FP receptor function upon stimulation with $PGF_{2\alpha}$ in accordance with the embodiment of the present invention;

Fig. 2A illustrates the effects of PCP-8 and PCP-10 on the diameter of the microvessels of pig retina upon stimulation with either $PGF_{2\alpha}$ or thromboxane $A_2$ mimetic, U46619;

Fig. 2B illustrates the dose response of $PGF_{2\alpha}$ on the diameter of pig microvessels treated previously with PCP-8 or PCP-10;

Fig. 2C illustrates the effects of thromboxane $A_2$ mimetic, U46619, on the diameter of pig microvessels treated previously with PCP-8 and PCP-10;

Fig. 3A illustrates the effects of PCP-10 upon spontaneous contractions of uterine smooth muscle;

Fig. 3B illustrates the dose response of prostaglandin $F_{2\alpha}$ in the presence/absence of PCP-8 and_PCP-10 upon uterine smooth muscle contraction; and

Fig. 4 illustrates the reversal of basal tone of bovine myometrium even in the presence of FP receptor ligand, $PGF_{2\alpha}$.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there is provided a new class of G protein-coupled receptor antagonists, which bind to the extracellular molecular surface, thus hamper signal transduction.

Also provided is a novel strategy to target the extracellular loops of the receptor which contribute to the structural or functional integrity of the receptor. Antagonists thus bind to cognate elements in the extracellular surface of the receptor and prevent the receptor function by interfering with its signal initiation or transduction.

There is provided proof of selectivity of the antagonists to FP receptor by showing an absence of their effects on a related prostanoid receptor for thromboxane $A_2$, known as TP receptor which is also involved in smooth muscle contraction.

**Preparation of inhibitors**

Chemical synthesis of PCP-8 and PCP-10:

All peptides which are 8 amino acids in length were synthesized using F-moc chemistry and solid phase Merrifield method two peptides, PCP-8 and PCP-10. These peptides were purified by HPLC and their purity tested by mass spectroscopy.

In accordance with the present invention, a novel strategy of using peptides derived from the extracellular domains of prostaglandin $F_{2\alpha}$ receptor, FP, to inhibit the signal transduction and the functional consequences of FP receptor. This method could be generalized to all G protein-coupled receptors. Peptides derived from the first and second extracellular loops of FP receptor were found to be effective inhibitors of FP receptor.

The present invention could be readily understood by referring to the following examples, which are given to illustrate the invention rather than to limit its scope.

## EXAMPLE I

### Effects of peptides, PCP-8 and PCP-10, on ligand-induced phosphoinositide hydrolysis in mammalian cells overexpressing the FP receptor

Both PCP-8 and -10 were tested in HEK293 cells expressing the human FP receptor. For this purpose, HEK 293 cells stably expressing human FP receptor were plated in 12-well plates in DMEM medium containing 10% fetal bovine serum, penicillin (10 U/ml) and streptomycin (10 $\mu$g/ml) and cultured in a humidified atmosphere containing 5% $CO_2$ at 37°C. After the wells were 80% confluent, the cells were labeled with 2 $\mu$Ci/ml of [$^3$H]-*myo* inositol overnight. Next day, the cells were washed once with PBS, and incubated in 0.5 ml of Kreb's buffer containing 10 mM LiCl and indicated concentrations of PCP peptides for 30 min. $PGF_{2\alpha}$ at 1$\mu$M was added to the cells and the incubation was carried out for an additional 30 min. The cells were solubilized with 0.1 N NaOH for 10 min and neutralized with 0.1 N formic acid. The lysates were collected and 1 ml each of methanol and chloroform were sequentially added and vortexed briefly. After centrifugation to separate the phases, inositol phosphates were separated by ion exchange chromatography as described below (Berridge, M.J. et al., 1983, *Biochem. J.* **212**:473-482).

Briefly, the medium was discarded and the IP3 synthesis was stopped by adding 0.6 ml ice-cold methanol. The cells were scraped and collected into polypropylene tubes. Distilled water (0.5 ml) and chloroform (0.6 ml) were added and vigorously vortexed for 2 min. The phases were separated by centrifugation at 6000 x g

for 10 min. The aqueous phase was applied to AG-1X-8™ (Formate form) anion exchange columns (1 ml bed volume) and free inositol was eluted with 10 ml of water, followed by 60 mM ammonium formate in 0.1 M formic acid. Then, the inositol phosphates were eluted with 5 ml of 1.2 M ammonium formate in 0.1 M formic acid. After adding 3 volumes of scintillation cocktail (Optiphase-HiSafe III), the eluates were counted by scintillation spectrophotometry.

The results of these experiments are shown in Fig. 1. Data are expressed as fold stimulation of inositol phosphate synthesis by 1 $\mu$M $PGF_{2\alpha}$ compared to the unstimulated controls. Both PCP-8 and -10 at 100 $\mu$M potently inhibited inositol phosphate synthesis initiated by the action of $PGF_{2\alpha}$ on FP receptor. The half maximal inhibitory concentrations for both PCP-8 and -10 were slightly less than 100 $\mu$M.

## EXAMPLE II

**Testing PCP peptides in porcine eyecup model of *ex vivo* vasomotricity assay**

In order to see if the peptides could inhibit FP function using an *ex vivo* model, we chose porcine eyecup model, an *ex vivo* assay of vascular constriction in porcine retinas which we previously described and validated (Li et al., 1996 J. Pharmacol. Expt. Therapeut. 278: 370-377; Li et al., 1997 Am. J. Physiol. 273: R1283-90; Abran et al., 1997 Am. J. Physiol. 272: R995-1001). Since FP receptor densities in newborn vasculature are minimal due to down regulation by high levels of circulating prostaglandins in the perinatal period, the newborn pigs were treated with a prostaglandin synthetase blocker, ibuprofen (30 mg/Kg of bodyweight/ 8 h for 24 h) to increase the density of the receptors as well as their vasomotor effects. By inhibiting circulating prostaglandins, we were able to show potent

inhibition of FP receptor-mediated second messenger synthesis as well as FP-mediated vascular constriction in this eyecup model.

To prepare eyecups, a circular incision was made 3-4 mm posterior to ora serrata to remove the interior segment and vitreous body with minimal handling of the retina. The remaining eyecup was fixed with pins to a wax base in a 20 ml tissue bath containing 20 ml of Kreb's buffer (pH 7.35-7.45), protease inhibitors, leupetin and aprotinin (10 $\mu$g/ml each), and equilibrated with 21% oxygen and 5% carbon dioxide at 37°C. The preparations were allowed to stabilize for 30 min. Peptides at 100 $\mu$M were added and incubation was continued for 30 min before the addition of $PGF_{2\alpha}$.

Cumulative concentration-responses of $PGF_{2\alpha}$ and $TxA_2$ mimetic, U46619, ($10^{-10}$ to $10^{-5}$ M) curves were constructed separately. To assess the reversibility of the antagonists, the eyecups were thoroughly washed (which would wash away the peptide) with incubation medium and concentration response curves for $PGF_{2\alpha}$ were determined. The outer vessel diameter was recorded with a video camera mounted on a dissecting microscope (Zeiss M 400™) and the responses were quantified by a digital image analyzer (Sigma Scan Software, Jandel Scientific, Corte Madera, CA). Vascular diameter was recorded before and 10 min following the topical application of the agent. Each measurement was repeated three times and showed <1% variability.

The results are shown in Fig. 2. The peptide PCP-10 had no effect on the basal tone (diameter of the microvessel) of the vessel (Fig. 2A; left panels). Addition of 1 $\mu$M of $PGF_{2\alpha}$ potently constricted the vessel in the absence of the peptide (middle-top panel), whereas presence of PCP-10 at 100 $\mu$M markedly inhibited $PGF_{2\alpha}$-mediated vasoconstriction (middle-bottom panel).

The peptide had no effect on the vasoconstriction effected by 1 $\mu$M $TxA_2$ mimetic, U46619, (right panels) acting on another prostanoid receptor coupled to constriction, namely TP receptor. Similar results were obtained for PCP-8 as well. A dose response of $PGF_{2\alpha}$ on the vascular diameter in the presence/absence of PCP-8 and PCP-10 peptides are presented in Fig. 2B. Both peptides abrogated the vasomotor responses even at concentrations exceeding 1$\mu$M of $PGF_{2\alpha}$, suggesting, as expected, that the peptides may be acting in a non-competitive fashion. However, the peptides had no effect on vasoconstriction produced by thromboxane $A_2$ (Fig. 2C).

Similarly, a peptide derived from the first extracellular loop of FP receptor, PCP-15, inhibited $PGF_{2\alpha}$-induced constriction ($10^{-7}$M) (88.1% over untreated control; Table 1).

## EXAMPLE III

### Testing peptide variants of PCP-8 in porcine eyecup model of ex vivo vasomotricity assay

In order to understand the structural requirements of PCP-8 in its inhibitory action on $PGF_{2\alpha}$-induced vasoconstriction, different amino acids in PCP-8 sequence were replaced with other D- or L- amino acids and the resulting peptides were chemically synthesized and tested in porcine eyecup model of *ex vivo* vasomotricity assay. These peptide variants are listed in Table 1.

Table 1

**Amino acid sequences of peptide variants of PCP-8 and their inhibitory potency in porcine eyecup model of *ex vivo* vasomotricity assay**

| Peptide PCP- | %Vasomotor response (of max. constriction)[1] | % inhibition of maximal response[2] | Peptide sequence | SEQ ID NO: |
|---|---|---|---|---|
| 8 | 50.0 | 50.0 | ilghrdyk | 1 |
| 10 | 20.0 | 80.0 | wedrfyll | 2 |
| 14 | 36.0 | 64.0 | YQDRFYLL | 3 |
| 13 | 20.0 | 80.0 | ILGHRDYK | 1 |
| 13.7 | 23.8 | 76.2 | ILAHRDYK | 4 |
| 13.8 | 46.8 | 53.2 | ILaHRDYK | 4 |
| 13.11 | 13.0 | 87.0 | ILGFRDYK | 5 |
| 13.13 | 36.9 | 63.1 | ILGHKDYK | 6 |
| 13.14 | 40.3 | 59.7 | ILGHRNYK | 7 |
| 13.18 | 30.0 | 70.0 | ILGHQDYK | 8 |
| 13.20 | 49.6 | 50.4 | ILGHRDY-amide | 9 |
| 13.21 | 46.2 | 53.8 | ILGHRDYK-amide | 1 |
| 13.22 | 16.6 | 83.4 | ILGWRDYK | 10 |
| 13.24 | 6.2 | 93.8 | ILGXRDYK | 11 |
| 15 | 11.9 | 88.1 | SNVLCSIF | 12 |

[1]Percent vasomotor response in the presence of 100 $\mu$M peptide is calculated as percent change in average vascular diameter produced by $10^{-7}$ M $PGF_{2\alpha}$ to the eyecup in the presence of the peptide compared to maximal constriction observed in the absence of the peptide.

[2]Percent inhibition produced by each peptide is calculated as (100-per cent vasomotor response).

Small letters indicate L-amino acids and capital letters indicate D- amino acids. I = isoleucine; L= leucine; G =glycine; H=histidine; R=Arginine; D=Aspartic acid; Y=Tyrosine; K=Lysine; A=Alanine; W= Tryptophan; E=Glutamic acid; F= Phenyl alanine; Q=Glutamine; N=Aspargine; P=Proline; S=Serine; X=Cyclohexyl alanine. Peptides were dissolved in DMSO freshly just before the experiment as 10 mM stocks and added to the eye cups 30 min before the addition of $10^{-7}$ M $PGF_{2\alpha}$.

A total of 25 variants of PCP-8 were synthesized and the efficacious or potent peptides are listed in Table 1. These peptides incorporate L- to D-amino acid changes, deletions, subtle variations in aromaticity, hydrogen bond donor status as opposed to ionic interactions and hydrophobicity. These peptides were tested at 100 μM concentration in porcine retinal vasomotricity assay and the results are summarized in Table 1.

The results are summarized as follows:

1. Converting all L-amino acids of PCP-8 to D-amino acids (PCP-13) increased the inhibitory potency dramatically. Removal of N-terminal hydrophobic dipeptide sequence from either PCP-8 (PCP-11) or PCP-10 (PCP-12) resulted in significant reduction in the inhibitory action of the peptides.
2. Glycine to alanine (13.7) does not change the activity of PCP-13, whereas change to proline (13.16), L-alanine (13.8), or deletion of the residue (13.17) entirely resulted in loss of activity. Glycine is an important linker residue separating the HRD motif from the IL hydrophobic sequence.
3. HRD-motif is important for the activity of PCP-13. Alanine substitutions (13.1-13.3) or to change to L-configuration (13.4-13.6) resulted loss of inhibitory activity of PCP-13. Aromaticity of His is more important than the positive charge, since H to F (13.11) or W (13.22) or X (13.24), but not to Y (13.23), did not result in significant reduction of peptide inhibitory potency. Side chain length appears to be more critical in case of D residue than R; changing D to E (13.12) resulted in loss of half of the inhibitory activity whereas R to K (13.13) or to Q (13.18) affected the activity of PCP-13 moderately. D to N (13.14) resulted in moderate loss of activity,

whereas a serine substitution (13.19) lead to drastic loss of activity of PCP-13.

4. Deletion of terminal lysine (13.15) or substitution with W (13.9) resulted in complete loss of activity; however, conversion of terminal carboxylate into an amide (13.20 & 13.21) resulted in moderate gain of activity of the peptide inhibitor. Substitution of aromatic residue, Y, with E (13.10) completely abolished the inhibitory potency of PCP-13.

Thus the structure of PCP-13 in D-configuration appears to consists of a N-terminal hydrophobic anchor spaced from the central HRD motif by a glycine residue possibly resulting in a turn conformation of the active peptide; Aromatic and hydrophobic interactions at the carboxy terminus may also add to the potency of PCP-13.

## EXAMPLE IV

### Testing PCP peptides in porcine uterine strip of *ex vivo* basal contraction assay

In *ex vivo* experiments using porcine uterine strips, the peptides were able to prevent both basal and $PGF_{2\alpha}$-induced contraction.

Uterine tissues from non-pregnant adult pigs were obtained from a local slaughter house and transported to the laboratory on ice. Uterine myometrial strips of approximately 1 cm in length were set up in organ baths containing Kreb's buffer equilibrated with 21% oxygen at 37°C as we have described (Potvin, W. et al., 1990, *Br. J. Pharmacol.* **100**:341-347; Varma, D.R. and Chemtob, S., 1993, *J. Pharmacol. Expt. Ther.* **265**:1096-1104). Contractions were recorded by force transducers on Grass-polygraph. Strips were incubated with or without 100 μM peptides for 30 min before adding $PGF_{2\alpha}$ in step-wise increments ($10^{-9}$ to $10^{-6}$ M). Data were expressed as percentage increase over the basal level of average tension (g).

A graph of spontaneous uterine contractions (known to be dependent upon prostanoids, mainly $PGF_{2\alpha}$) in the absence and the presence of 100 μM PCP-8 are shown in Fig. 3A. Addition of peptide to the strips reduced the force of basal contraction. A dose response of $PGF_{2\alpha}$ on uterine contractility in the presence or absence of PCP-8 and PCP-10 peptides is shown in Fig. 3B. More than 60% (PCP-8) and 80% (PCP-10) reduction in uterine contraction was observed in all concentrations of $PGF_{2\alpha}$ tested. Thus, both these peptides reduced spontaneous as well as $PGF_{2\alpha}$-induced contractions in the uterine strips.

## EXAMPLE V

**Testing PCP peptides in bovine uterine strip of *ex vivo* basal contraction assay**

Uterine tissues from non-pregnant adult bovine animals were obtained from a local slaughter house and transported to the laboratory on ice. Uterine myometrial strips of approximately 1 cm in length were set up in organ baths containing Kreb´s buffer equilibrated with 21% oxygen at 37°C as described above. Contractions were recorded on Grass-polygraph by force transducers. Strips were incubated with or without 100 μM peptides before adding $PGF_{2\alpha}$ in step-wise increments ($10^{-8}$ to $10^{-6}$ M). Data were expressed as change in basal level of average tension (g). The results are shown in Fig. 4. Application of PCP-10 peptide at 100 μM reversed the basal tone (contractile state) of the uterine muscle. Addition of $PGF_{2\alpha}$ up to 10μM did not affect the relaxation produced by PCP-10 suggesting that the effects of PCP peptides are independent of the ligand, which was also shown in the previous results.

While the invention has been described in connection with specific embodiment thereof, it will be understood that it is capable of further modifications

and this application is intended to cover any variations, uses or adaptations of the invention following in general, the principles of the invention and including such departures from the present disclosure as come within the known customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

**WHAT IS CLAIMED IS:**

1. A G protein-coupled receptor agonist or antagonist which specifically binds to the juxtamembrane extracellular structural elements of the G protein-coupled receptor in a manner different from that of the natural ligand, and wherein said agonist or antagonist alters the transduction of an intracellular signal.

2. The agonist or antagonist of claim 1, wherein said agonist or antagonist does not crossreact with other prostanoid receptors and wherein said antagonist is effective in the presence of excess ligand.

3. The agonist or antagonist of claim 1, which comprises an amino acid sequence derived from derived from the first and/or second extracellular loops of prostanoid receptors.

4. The agonist or antagonist of claim 3, which comprises an amino acid sequence derived from the first and second extracellular loop of prostanoid receptors.

5. The antagonist of claim 1, wherein the receptor is prostaglandin $F_{2\alpha}$ receptor (FP receptor).

6. The antagonist of claim 2, which comprises amino acid sequence of the FP receptor selected from the group consisting of ILGHRDYK (PCP-8; SEQ ID NO:1); WEDRFYLL (PCP-10; SEQ ID NO:2); YQDRFYLL (PCP-14; SEQ ID NO:3); ILAHRDYK (PCP-13.7; SEQ ID NO:4); ILGFRDYK (PCP-13.11; SEQ ID NO:5); ILGHKDYK (PCP-13.13; SEQ ID NO:6); ILGHRNYK (PCP-13.14; SEQ ID NO:7); ILGHQDYK (PCP-13.18; SEQ ID NO:8); ILGHRDY-amide (PCP-13.20; SEQ ID NO:9); ILGHRDYK-amide (PCP-13.21; SEQ ID NO:1);

ILGWRDYK (PCP-13.22; SEQ ID NO:10); ILGXRDYK (PCP-13.24; SEQ ID NO:11); SNVLCSIF (PCP-15; SEQ ID NO:12); protein fusions and peptidomimetics thereof; wherein said amino acid sequence contains L- and/or D-amino acid.

7. A peptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1 to 12 and wherein said amino acid sequence contains L- and/or D-amino acid, an amino acid sequence with at least about 90% homology to SEQ ID NO:1 to 12, and peptidomimetic thereof.

8. A method for preventing premature delivery of fetus, which comprises the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist of any one of claims 1 to 6 or functional derivatives thereof.

9. A method for preventing and/or treating dysmenorrhea comprising the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist of any one of claims 1 to 6 or functional derivatives thereof.

10. A pharmaceutical composition containing at least a G protein-coupled receptor agonist of any one of claims 1 to 4, an antagonist of any one of claims 1 to 6, mixture thereof, or functional derivatives thereof in association with a pharmaceutically acceptable carrier.

11. A method of identifying a compound as a G protein-coupled receptor agonist or antagonist capable of binding to the extracellular elements of the said receptor in a manner different from that of the natural ligand, comprising the steps of:

a) culturing cells which express said receptor or identifying animal tissues *ex vivo* or *in vivo* where physiological consequences are dependent on said receptor;

b) contacting said cells or tissues with said compound to be tested for agonist or antagonist activity at said receptor; and

d) measuring a response to alter the transduction of a signal resulting in physiological consequences selected from the group consisting of increments in cell calcium, phosphoinositide hydrolysis, increased/decreased cellular cyclic adenosine monophosphate, cell growth and/or differentiation, altered gene expression, and smooth muscle contraction or dilation, wherein said response is indicative of agonist or antagonist activity.

12. A method of identifying a compound as a prostaglandin $F_2$ alpha receptor agonist or antagonist capable of binding to the extracellular elements of the said receptor in a manner different from that of the natural ligand, comprising the steps of:

a) culturing cells which express said receptor or identifying animal tissues *ex vivo* or *in vivo* where physiological consequences are dependent on said receptor;

b) contacting said cells or tissues with said compound to be tested for agonist or antagonist activity at said receptor; and

c) measuring a response to alter the transduction of a signal resulting in physiological consequences selected from the group consisting of increments in cell calcium, phosphoinositide hydrolysis, cell growth and/or differentiation, altered gene expression, and smooth muscle contraction or dilation, wherein said response is indicative of agonist or antagonist activity.

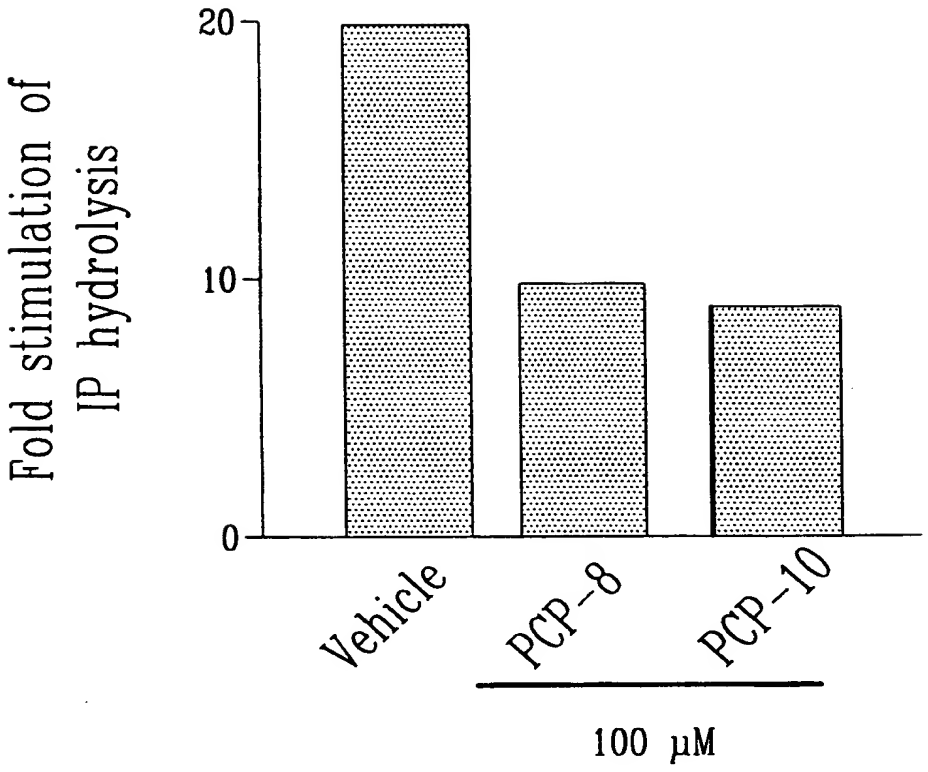

Fold stimulation of IP hydrolysis
20
10
0
Vehicle
PCP-8
PCP-10
100 µM

Fig. 1

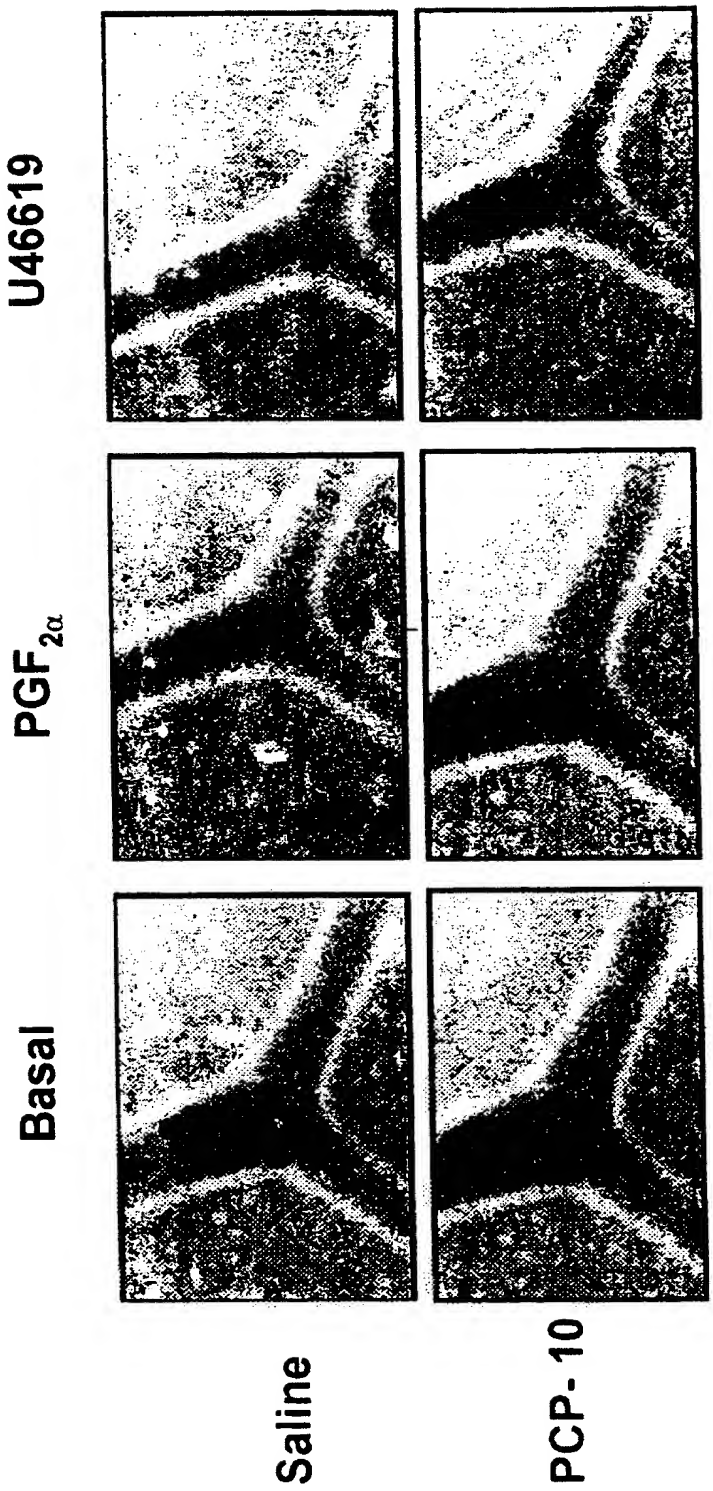



FIG - 2B

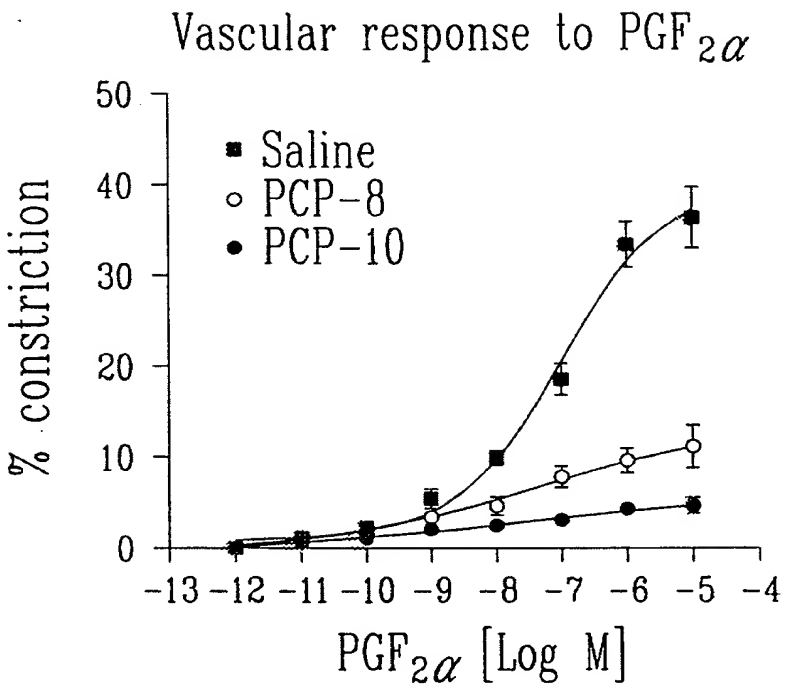

Vascular response to $PGF_{2\alpha}$
% constriction
50
40
30
20
10
0
Saline
PCP-8
PCP-10
-13 -12 -11 -10 -9 -8 -7 -6 -5 -4
$PGF_{2\alpha}$ [Log M]

Fig 2b

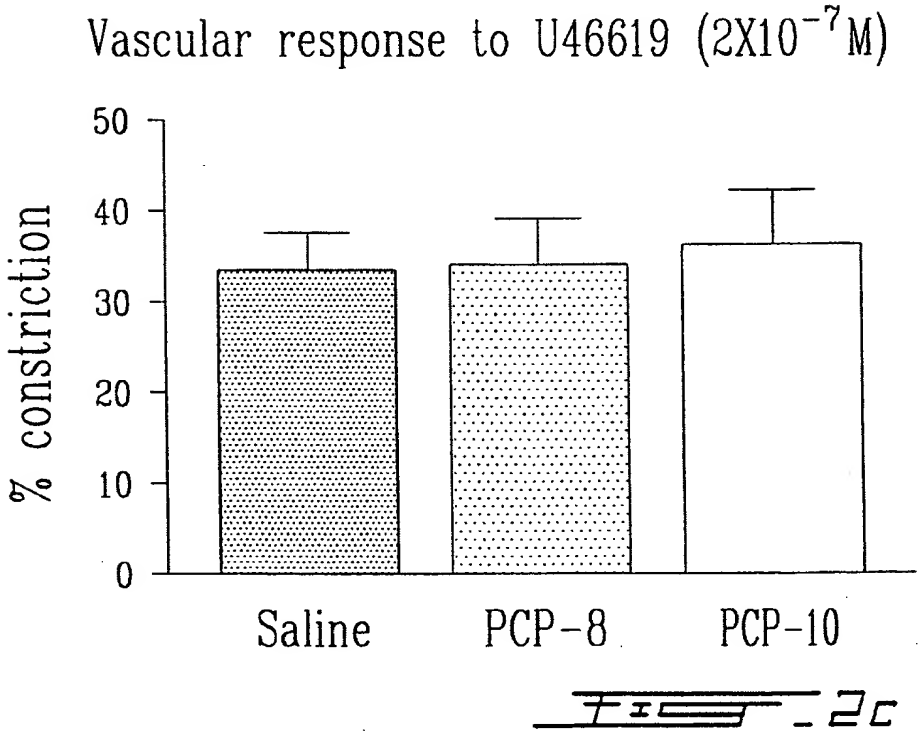

Vascular response to U46619 ($2X10^{-7}$M)
% constriction
50
40
30
20
10
0
Saline
PCP-8
PCP-10

Fig 2c

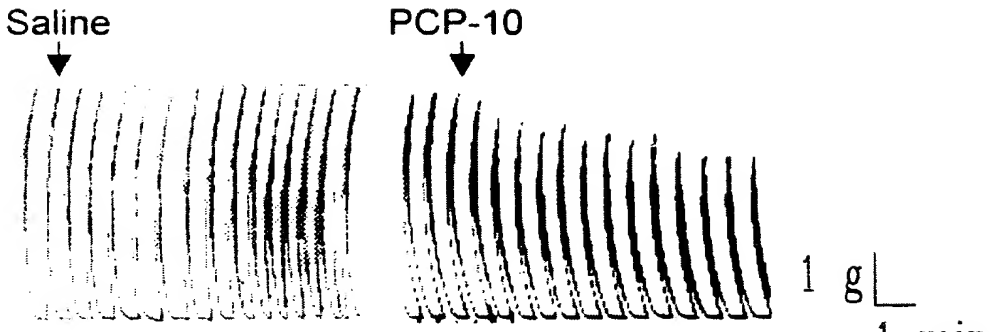



Fig 3a

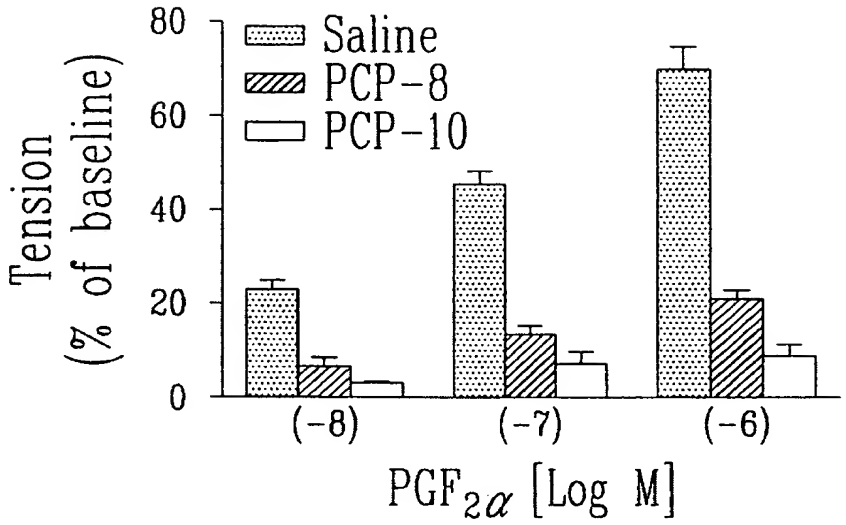

Tension
(% of baseline)
80
60
40
20
0
Saline
PCP-8
PCP-10
(-8)
(-7)
(-6)
PGF2α [Log M]

Fig 3b

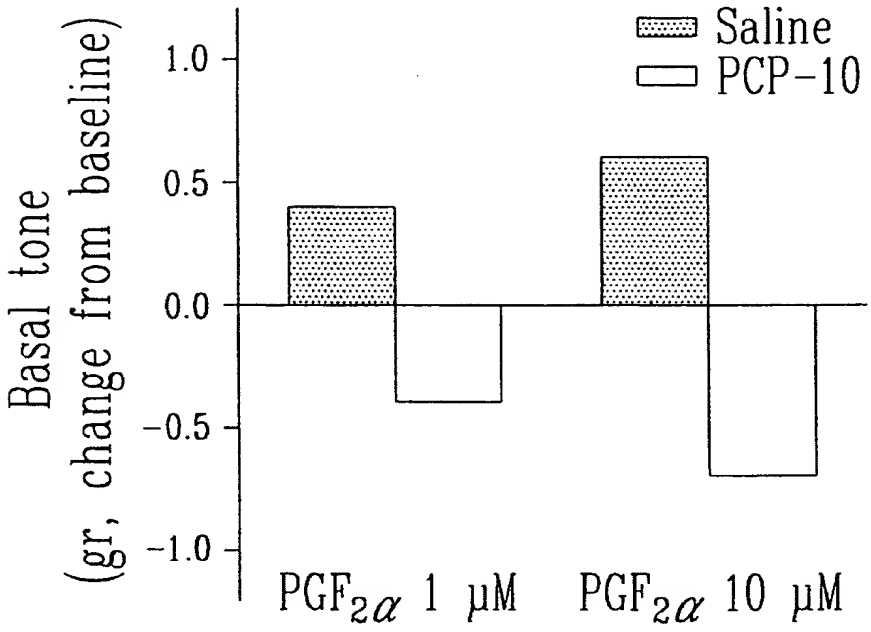

Basal tone
(gr, change from baseline)
1.0
0.5
0.0
-0.5
-1.0
Saline
PCP-10
PGF2α 1 μM
PGF2α 10 μM

Fig 4

SEQUENCE LISTING

<110> HÔPITAL SAINTE-JUSTINE
CHEMTOB, Sylvain
PERI, Krishna G.

<120> G PROTEIN-COUPLED RECEPTOR ANTAGONISTS

<130> 12667-16PCT FC/ld

<150> US 09/154,627
<151> 1998-09-17

<160> 12

<170> FastSEQ for Windows Version 3.0

<210> 1
<211> 8
<212> PRT
<213> FP receptor peptides

<400> 1
Ile Leu Gly His Arg Asp Tyr Lys
1 5

<210> 2
<211> 8
<212> PRT
<213> FP receptor peptides

<400> 2
Trp Glu Asp Arg Phe Tyr Leu Leu
1 5

<210> 3
<211> 8
<212> PRT
<213> FP receptor peptides

<400> 3
Tyr Gln Asp Arg Phe Tyr Leu Leu
1 5

<210> 4
<211> 8
<212> PRT
<213> FP receptor peptides

<400> 4
Ile Leu Ala His Arg Asp Tyr Lys
1 5

<210> 5
<211> 8
<212> PRT
<213> FP receptor peptides

<400> 5
Ile Leu Gly Phe Arg Asp Tyr Lys
1 5

<210> 6
<211> 8
<212> PRT
<213> FP receptor peptides

<400> 6
Ile Leu Gly His Lys Asp Tyr Lys
1 5

<210> 7
<211> 8
<212> PRT
<213> FP receptor peptides

<400> 7
Ile Leu Gly His Arg Asn Tyr Lys
1 5

<210> 8
<211> 8
<212> PRT
<213> FP receptor peptides

<400> 8
Ile Leu Gly His Gln Asp Tyr Lys
1 5

<210> 9
<211> 7
<212> PRT
<213> FP receptor peptides

<400> 9
Ile Leu Gly His Arg Asp Tyr
1 5

<210> 10
<211> 8
<212> PRT
<213> FP receptor peptides

<400> 10
Ile Leu Gly Trp Arg Asp Tyr Lys
1 5

<210> 11
<211> 8
<212> PRT
<213> FP receptor peptides

<400> 11
Ile Leu Gly Xaa Arg Asp Tyr Lys
1 5

<210> 12
<211> 8
<212> PRT
<213> FP receptor peptides

<400> 12
Ser Asn Val Leu Cys Ser Ile Phe
1 5

# INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/CA 99/00844

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/705 C07K14/72 A61K38/04 A61P15/06
G01N33/50 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N A61K A61P G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>A | WO 95 00551 A (MERCK FROSST CANADA INC ;ABRAMOVITZ MARK (CA); GRYGORCZYK RICHARD) 5 January 1995 (1995-01-05)<br>page 14, line 17 - line 29; claim 8; figures 3,5,6 | 1,7, 10-12<br>2-6,8,9 |
| X | WO 96 23225 A (COR THERAPEUTICS INC) 1 August 1996 (1996-08-01)<br>page 17, paragraph 5<br>page 23<br>claims 7-21; examples 6,7,11 | 1-4,10, 11 |
| X | WO 93 09104 A (SEARLE & CO) 13 May 1993 (1993-05-13)<br>page 8, line 6 - line 19<br>page 19, line 1 - line 17<br>page 61 -page 62<br>claims 36,37 | 1,2,9-11 |

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 10 February 2000 | 01/03/2000 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31–70) 340–2040, Tx. 31 651 epo nl,<br>Fax: (+31–70) 340–3016 | van Klompenburg, W |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Intern. al Application No
PCT/CA 99/00844

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 5 688 938 A (BROWN EDWARD M ET AL)<br>18 November 1997 (1997-11-18)<br>column 4, line 23 - line 36<br>column 22, line 48 -column 44, line 28<br>examples 1-14; tables 2-8<br>--- | 1,2,10,<br>11 |
| X | KITANAKA ET AL.: "Phloretin as an antagonist of prostaglandin F2alpha receptor in cultured rat astrocytes"<br>JOURNAL OF NEUROCHEMISTRY,<br>vol. 60, no. 2, February 1993 (1993-02),<br>pages 704-708, XP000876615<br>page 704<br>page 707 -page 708; figures 1-5; table 1<br>--- | 1,5,<br>10-12 |
| A | SUGIMOTO ET AL.: "Failure of parturition in mice lacking the prostaglandin f receptor"<br>SCIENCE,<br>vol. 277, 1 August 1997 (1997-08-01),<br>pages 681-683, XP002130206<br>cited in the application<br>page 681 -page 683; figure 2<br>--- | 8 |
| P,X | GRIFFIN B W ET AL: "AL-8810: a novel prostaglandin F2 alpha analog with selective antagonist effects at the prostaglandin F2 alpha ( FP ) receptor."<br>JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, (1999 SEP) 290 (3) 1278-84.<br>, XP000876534<br>page 1282, column 2 -page 1283, column 1;<br>figures 2-8<br>----- | 1,2,5,<br>8-12 |

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 99/ 00844

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 8 and 9 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern[illegible]al Application No
PCT/CA 99/00844

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9500551 A | 05-01-1995 | US 5869281 A | 09-02-1999 |
| | | AT 170532 T | 15-09-1998 |
| | | AU 6991894 A | 17-01-1995 |
| | | CA 2165954 A | 05-01-1995 |
| | | DE 69413039 D | 08-10-1998 |
| | | DE 69413039 T | 06-05-1999 |
| | | EP 0705276 A | 10-04-1996 |
| | | ES 2120052 T | 16-10-1998 |
| | | JP 11505701 T | 25-05-1999 |
| | | US 5840847 A | 24-11-1998 |
| WO 9623225 A | 01-08-1996 | AU 2397399 A | 17-06-1999 |
| | | AU 701822 B | 04-02-1999 |
| | | AU 4859996 A | 14-08-1996 |
| | | CA 2211176 A | 01-08-1996 |
| | | EP 0870198 A | 14-10-1998 |
| | | JP 11514207 T | 07-12-1999 |
| | | NO 973422 A | 24-09-1997 |
| | | NZ 302524 A | 28-05-1999 |
| | | US 5763575 A | 09-06-1998 |
| | | US 5716789 A | 10-02-1998 |
| | | US 5874400 A | 23-02-1999 |
| WO 9309104 A | 13-05-1993 | AU 2669992 A | 07-06-1993 |
| | | CA 2118688 A | 13-05-1993 |
| | | EP 0613472 A | 07-09-1994 |
| | | JP 7501054 T | 02-02-1995 |
| | | US 5449674 A | 12-09-1995 |
| US 5688938 A | 18-11-1997 | US 6011068 A | 04-01-2000 |
| | | AU 709303 B | 26-08-1999 |
| | | AU 4195796 A | 15-05-1996 |
| | | BR 9509411 A | 30-12-1997 |
| | | CA 2202879 A | 02-05-1996 |
| | | CN 1220658 A | 23-06-1999 |
| | | CZ 9701182 A | 17-09-1997 |
| | | EP 0787122 A | 06-08-1997 |
| | | HU 77980 A | 01-02-1999 |
| | | JP 11130737 A | 18-05-1999 |
| | | JP 2882882 B | 12-04-1999 |
| | | JP 10513436 T | 22-12-1998 |
| | | NZ 297157 A | 30-08-1999 |
| | | PL 319812 A | 01-09-1997 |
| | | WO 9612697 A | 02-05-1996 |
| | | US 6001884 A | 14-12-1999 |
| | | US 5858684 A | 12-01-1999 |
| | | US 5763569 A | 09-06-1998 |
| | | US 5962314 A | 05-10-1999 |
| | | WO 9511221 A | 27-04-1900 |
| | | AU 3122699 A | 22-07-1999 |
| | | AU 702629 B | 25-02-1999 |
| | | AU 8087294 A | 08-05-1995 |
| | | CA 2173747 A | 27-04-1995 |
| | | CN 1139917 A | 08-01-1997 |
| | | EP 0724561 A | 07-08-1996 |
| | | JP 9504032 T | 22-04-1997 |
| | | SG 52796 A | 28-09-1998 |
| | | WO 9304373 A | 04-03-1993 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No
PCT/CA 99/00844

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| US 5688938 A | | AU 673500 B | 14-11-1996 |
| | | AU 711247 B | 07-10-1999 |
| | | AU 7197796 A | 20-02-1997 |
| | | CA 2115828 A | 04-03-1993 |
| | | CN 1071333 A | 28-04-1993 |
| | | EP 0657029 A | 14-06-1995 |
| | | JP 2860285 B | 24-02-1999 |
| | | JP 9328420 A | 22-12-1997 |
| | | JP 2887201 B | 26-04-1999 |
| | | JP 9281109 A | 31-10-1997 |
| | | JP 11221095 A | 17-08-1999 |
| | | JP 2728564 B | 18-03-1998 |
| | | JP 6510531 T | 24-11-1994 |
| | | MX 9204881 A | 01-04-1993 |
| | | NO 940581 A | 25-04-1994 |
| | | ZA 9206360 A | 30-03-1993 |

PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference | FOR FURTHER ACTION | see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. |
|---|---|---|
| 12667-16PCT | | |
| International application No. | International filing date *(day/month/year)* | (Earliest) Priority Date *(day/month/year)* |
| PCT/CA 99/ 00844 | 15/09/1999 | 17/09/1998 |
| Applicant | | |
| HÔPITAL SAINTE-JUSTINE et al. | | |

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [X] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rul 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [ ] as suggested by the applicant.

   [X] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 99/ 00844

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 8 and 9 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# PATENT COOPERATION TREATY



From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Côté, France
SWABEY OGILVY RENAULT
1981 McGill College Avenue
Suite 1600
Montréal, Québec H3A 2Y3
CANADA

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

| | |
|---|---|
| Date of mailing *(day/month/year)* | 27. 10. 00 |
| Applicant's or agent's file reference<br>12667-16PCT | **IMPORTANT NOTIFICATION** |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/CA99/00844 | 15/09/1999 | 17/09/1998 |

Applicant
HÔPITAL SAINTE-JUSTINE et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

| Name and mailing address of the IPEA/ | Authorized officer |
|---|---|
| European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Emslander, S<br><br>Tel.+49 89 2399-8718 |

Form PCT/IPEA/416 (July 1992)

ATENT COOPERATION T[redacted]ATY

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| 12667-16PCT | | |
| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
| PCT/CA99/00844 | 15/09/1999 | 17/09/1998 |
| International Patent Classification (IPC) or national classification and IPC<br>C12N15/12 | | |
| Applicant<br>HÔPITAL SAINTE-JUSTINE et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 10/04/2000 | 27. 10. 00 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized officer<br>Heimann-Pohl, B<br>Telephone No. +49 89 2399 8713 |

Form PCT/IPEA/409 (cover sheet) (January 1994)

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA99/00844

## I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

**Description, pages:**

| | | |
|---|---|---|
| 2-18 | as originally filed | |
| 1 | with telefax of | 24/10/2000 |

**Claims, No.:**

| | | |
|---|---|---|
| 1-9 | with telefax of | 24/10/2000 |

**Drawings, sheets:**

| | |
|---|---|
| 1/5-5/5 | as originally filed |

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have be considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

## II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

☐ copy of the earlier application whose priority has been claimed.

☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

**see separate sheet**

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-9 |
| | No: | Claims | |
| Inventive step (IS) | Yes: | Claims | 1-9 |
| | No: | Claims | |
| Industrial applicability (IA) | Yes: | Claims | 1,2,5-9 |
| | No: | Claims | |

2. Citations and explanations

**see separate sheet**

1). The present application relates to G protein-coupled receptor agonists and antagonists. More specifically the present application provides peptide antagonists for FP receptor which peptides comprise an amino acid sequence derived from FP receptor.

2). Prior Art

D1: WO 95 00551 A (MERCK FROSST CANADA INC ;ABRAMOVITZ MARK (CA); GRYGORCZYK RICHARD) 5 January 1995 (1995-01-05)
D2: WO 96 23225 A (COR THERAPEUTICS INC) 1 August 1996 (1996-08-01)
D3: WO 93 09104 A (SEARLE & CO) 13 May 1993 (1993-05-13)
D4: US-A-5 688 938 (BROWN EDWARD M ET AL) 18 November 1997 (1997-11-18)
D5: KITANAKA ET AL.: 'Phloretin as an antagonist of prostaglandin F2alpha receptor in cultured rat astrocytes' JOURNAL OF NEUROCHEMISTRY, vol. 60, no. 2, February 1993 (1993-02), pages 704-708, XP000876615
D6: SUGIMOTO ET AL.: 'Failure of parturition in mice lacking the prostaglandin f receptor' SCIENCE, vol. 277, 1 August 1997 (1997-08-01), pages 681-683, XP002130206 cited in the application
D7: GRIFFIN B W ET AL: 'AL-8810: a novel prostaglandin F2 alpha analog with selective antagonist effects at the prostaglandin F2 alpha ( FP ) receptor.' JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, (1999 SEP) 290 (3) 1278-84. , XP000876534

D1 discloses the sequence encoding FP receptor (Table 2, page 17, Fig. 2). D1 refers to the uses of potential agonists and antagonists e.g. for treatment of glaucoma and dysmenorrhoea (page 14). Further the known agonist fluprostenol is used (page 21).

D2 concerns G protein-coupled receptor related to the thrombin receptor. D2 discloses agonist peptides i.a. those from the extracellular loops and the use of antibodies as antagonists which are directed against portions of the sequence which form the extracellular loops (page 16).

D3 relates to prostaglandin antagonists (small chemical molecules) and their use

for treatment of dysmenorrhoea.

D4 relates to calcium receptors and modulating agent therefore.

D5 describes the glucose transport inhibitor Phloretin to act as antagonist of $PGF_{2\alpha}$ by interfering with $PGF_{2\alpha}$ specific receptors in cultured rat astrocytes.

D6 shows that $PGF_{2\alpha}$ induces parturition upon interaction with FP receptor.

3). Novelty (Art. 33 (2) PCT) and Inventive Step (Art. 33 (3) PCT) (Box V)

None of the available prior art documents (D1-D7) discloses antagonists derived from a sequence of about 6-12 amino acids selected from the junction between a transmembrane segment and an adjoining extracellular loop of a G protein-coupled receptor, specifically SEQ ID NOs 1-12. Therefore the subject matter of claims 1-9 appears to be novel.
Since no indications were found in the prior art (D1-D7) that peptides derived from said sequence could be acting as antagonists, they appear not obvious, thus an inventive step can be acknowledged for claims 1-9.

4). Industrial Applicability (Box V)

For the assessment of the present claims 3 and 4 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

5). Priority

If the priority is valid for SEQ ID NO 1 and 2.
Document D7, is not relevant to the claimed subject matter since AL-8810 is not a peptide.

6). The Sequence Listing has been taken into account.

7). The reference to claim 3 in claim 1 is apparently wrong, due to an obvious editorial error occurred during amendment of said claim. Thus claim 1 is read as "A G protein-coupled receptor antagonist which comprises...".

15

# PATENT COOPERATION TREATY

# PCT

REC'D 31 OCT 2000
WIPO PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| 12667-16PCT | | |
| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
| PCT/CA99/00844 | 15/09/1999 | 17/09/1998 |
| International Patent Classification (IPC) or national classification and IPC<br>C12N15/12 | | |
| Applicant<br>HÔPITAL SAINTE-JUSTINE et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 10/04/2000 | 27.10.00 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized officer<br>Heimann-Pohl, B<br>Telephone No. +49 89 2399 8713 |

Form PCT/IPEA/409 (cover sheet) (January 1994)

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA99/00844

## I. Basis f th r port

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

**Description, pages:**

| | | |
|---|---|---|
| 2-18 | as originally filed | |
| 1 | with telefax of | 24/10/2000 |

**Claims, No.:**

| | | |
|---|---|---|
| 1-9 | with telefax of | 24/10/2000 |

**Drawings, sheets:**

| | |
|---|---|
| 1/5-5/5 | as originally filed |

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

## II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

☐ copy of the earlier application whose priority has been claimed.

☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

**s e separate sheet**

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-9 |
| | No: | Claims | |
| Inventive step (IS) | Yes: | Claims | 1-9 |
| | No: | Claims | |
| Industrial applicability (IA) | Yes: | Claims | 1,2,5-9 |
| | No: | Claims | |

2. Citations and explanations

**see separate sheet**

1). The present application relates to G protein-coupled receptor agonists and antagonists. More specifically the present application provides peptide antagonists for FP receptor which peptides comprise an amino acid sequence derived from FP receptor.

2). Prior Art

D1: WO 95 00551 A (MERCK FROSST CANADA INC ;ABRAMOVITZ MARK (CA); GRYGORCZYK RICHARD) 5 January 1995 (1995-01-05)
D2: WO 96 23225 A (COR THERAPEUTICS INC) 1 August 1996 (1996-08-01)
D3: WO 93 09104 A (SEARLE & CO) 13 May 1993 (1993-05-13)
D4: US-A-5 688 938 (BROWN EDWARD M ET AL) 18 November 1997 (1997-11-18)
D5: KITANAKA ET AL.: 'Phloretin as an antagonist of prostaglandin F2alpha receptor in cultured rat astrocytes' JOURNAL OF NEUROCHEMISTRY, vol. 60, no. 2, February 1993 (1993-02), pages 704-708, XP000876615
D6: SUGIMOTO ET AL.: 'Failure of parturition in mice lacking the prostaglandin f receptor' SCIENCE, vol. 277, 1 August 1997 (1997-08-01), pages 681-683, XP002130206 cited in the application
D7: GRIFFIN B W ET AL: 'AL-8810: a novel prostaglandin F2 alpha analog with selective antagonist effects at the prostaglandin F2 alpha ( FP ) receptor.' JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, (1999 SEP) 290 (3) 1278-84. , XP000876534

D1 discloses the sequence encoding FP receptor (Table 2, page 17, Fig. 2). D1 refers to the uses of potential agonists and antagonists e.g. for treatment of glaucoma and dysmenorrhoea (page 14). Further the known agonist fluprostenol is used (page 21).

D2 concerns G protein-coupled receptor related to the thrombin receptor. D2 discloses agonist peptides i.a. those from the extracellular loops and the use of antibodies as antagonists which are directed against portions of the sequence which form the extracellular loops (page 16).

D3 relates to prostaglandin antagonists (small chemical molecules) and their use

for treatment of dysmenorrhoea.

D4 relates to calcium receptors and modulating agent therefore.

D5 describes the glucose transport inhibitor Phloretin to act as antagonist of $PGF_{2\alpha}$ by interfering with $PGF_{2\alpha}$ specific receptors in cultured rat astrocytes.

D6 shows that $PGF_{2\alpha}$ induces parturition upon interaction with FP receptor.

3). Novelty (Art. 33 (2) PCT) and Inventive Step (Art. 33 (3) PCT) (Box V)

None of the available prior art documents (D1-D7) discloses antagonists derived from a sequence of about 6-12 amino acids selected from the junction between a transmembrane segment and an adjoining extracellular loop of a G protein-coupled receptor, specifically SEQ ID NOs 1-12. Therefore the subject matter of claims 1-9 appears to be novel.
Since no indications were found in the prior art (D1-D7) that peptides derived from said sequence could be acting as antagonists, they appear not obvious, thus an inventive step can be acknowledged for claims 1-9.

4). Industrial Applicability (Box V)

For the assessment of the present claims 3 and 4 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

5). Priority

If the priority is valid for SEQ ID NO 1 and 2.
Document D7, is not relevant to the claimed subject matter since AL-8810 is not a peptide.

6). The Sequence Listing has been taken into account.

7). The reference to claim 3 in claim 1 is apparently wrong, due to an obvious editorial error occurred during amendment of said claim. Thus claim 1 is read as "A G protein-coupled receptor antagonist which comprises...".

# G PROTEIN-COUPLED RECEPTOR ANTAGONISTS

## BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to development of agonist or antagonist of a G protein-coupled receptor, which bind to the G protein-coupled receptor from the extracellular side in a manner different from that of the natural ligand.

(b) Description of Prior Art

Prostaglandins are derived from the oxygenation of arachidonic acid by prostaglandin synthetases. Prostaglandins mediate a wide variety of physiological actions, such as vasomotricity, sleep/wake cycle, intestinal secretion, lipolysis, glomelular filtration, mast cell degranulation, neurotransmission, platelet aggregation, leuteolysis, myometrial contraction and labor, inflammation and arthritis, patent ductus arteriosus, cell growth and differentiation (Coleman, R.A. et al., 1994, *Pharmacol. Rev.* 46:205-229; Goetzl, E.J. et al., 1995, *FASEB J.* 9:1051-10585). Prostanoids mediate their actions through binding to distinct receptors, which belong to the super family of rhodopsin-like seven transmembrane helical receptors. These receptors are coupled to heterotrimeric G-proteins comprising of $\alpha$, $\beta$ and $\gamma$ subunits which, upon activation, elicit alterations in cell calcium, initiate phosphoinositide hydrolysis or promotion or repression of cyclic adenosine monophosphate synthesis (Strader C. D. et al., 1994, *Ann. Rev. Biochem.* 63:101-132).

Of the five pharmacologically distinct prostanoid receptors for $PGE_2$, $PGI_2$, $PGD_2$, $PGF_{2\alpha}$ and $TxA_2$ and their many isoforms, the receptor for $PGF_{2\alpha}$, also called FP receptor, shows limited tissue distribution, predominantly expressed in corpora leutea, uterine myome-

**WHAT IS CLAIMED IS:**

1. A G protein-coupled receptor antagonist of claim 3, which comprises amino acid sequence of the FP receptor selected from the group consisting of ILGHRDYK (PCP-8; SEQ ID NO:1); WEDRFYLL (PCP-10; SEQ ID NO:2); YQDRFYLL (PCP-14; SEQ ID NO:3); ILAHRDYK (PCP-13.7; SEQ ID NO:4); ILGFRDYK (PCP-13.11; SEQ ID NO:5); ILGHKDYK (PCP-13.13; SEQ ID NO:6); ILGHRNYK (PCP-13.14; SEQ ID NO:7); ILGHQDYK (PCP-13.18; SEQ ID NO:8); ILGHRDY-amide (PCP-13.20; SEQ ID NO:9); ILGHRDYK-amide (PCP-13.21; SEQ ID NO:1); ILGWRDYK (PCP-13.22; SEQ ID NO:10); ILGXRDYK (PCP-13.24; SEQ ID NO:11); SNVLCSIF (PCP-15; SEQ ID NO:12); and functional peptide analogues thereof, wherein X is cyclohexyl alanine.

2. A peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NO:1 to 12 and wherein said amino acid sequence contains L- and/or D-amino acid, an amino acid sequence with at least about 90% homology to SEQ ID NO:1 to 12.

3. A method for preventing premature delivery of fetus, which comprises the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist of claim 1.

4. A method for preventing and/or treating dysmenorrhea comprising the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist of claim 1.

5. A pharmaceutical composition containing at least a G protein-coupled receptor an antagonist of claim 1, mixture thereof, in association with a pharmaceutically acceptable carrier.

6. A method for determining activity of a compound of claim 1 as a G protein-coupled receptor antagonist capable of binding to the extracellular elements of the said receptor, comprising the steps of:

a) culturing cells which express said receptor or identifying animal tissues *ex vivo* or *in vivo* where physiological consequences are dependent on said receptor;

b) contacting said cells or tissues with said compound at a concentration of $10^{-10}$ M to $10^{-3}$ M to be tested for antagonist activity at said receptor; and

c) measuring a response to alter the transduction of a signal resulting in physiological consequences selected from the group consisting of increments in cell calcium, phosphoinositide hydrolysis, increased/decreased cellular cyclic adenosine monophosphate, cell growth and/or differentiation, altered gene expression, and smooth muscle contraction or dilation.

7. A method for determining activity of a compound of claim 1 as a prostaglandin $F_2$ alpha receptor antagonist capable of binding to the extracellular elements of the said receptor, comprising the steps of:

a) culturing cells which express said receptor or identifying animal tissues *ex vivo* or *in vivo* where physiological consequences are dependent on said receptor;

b) contacting said cells or tissues with said compound at a concentration of $10^{-10}$ M to $10^{-3}$ M to be tested for antagonist activity at said receptor; and

c) measuring a response to alter the transduction of a signal resulting in physiological consequences selected from the group consisting of increments in cell calcium, phosphoinositide hydrolysis, cell growth and/or differentiation, altered gene expression, and smooth muscle contraction or dilation.

8. The use of a therapeutically effective amount of a G protein-coupled receptor antagonist of claim 1 ~~4~~ for the preparation of a medicament for preventing premature delivery of fetus.

9. The use of a therapeutically effective amount of a G protein-coupled receptor antagonist of claim 1 ~~4~~ for the preparation of a medicament for preventing and/or treating dysmenorrhea.

# G PROTEIN-COUPLED RECEPTOR AGONISTS OR ANTAGONISTS

## BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to development of agonist or antagonist of a G protein-coupled receptor, which bind to the G protein-coupled receptor from the extracellular side in a manner different from that of the natural ligand.

(b) Description of Prior Art

Prostaglandins are derived from the oxygenation of arachidonic acid by prostaglandin synthetases. Prostaglandins mediate a wide variety of physiological actions, such as vasomotricity, sleep/wake cycle, intestinal secretion, lipolysis, glomelular filtration, mast cell degranulation, neurotransmission, platelet aggregation, leuteolysis, myometrial contraction and labor, inflammation and arthritis, patent ductus arteriosus, cell growth and differentiation (Coleman, R.A. et al., 1994, *Pharmacol. Rev.* **46**:205-229; Goetzl, E.J. et al., 1995, *FASEB J.* **9**:1051-10585). Prostanoids mediate their actions through binding to distinct receptors, which belong to the super family of rhodopsin-like seven transmembrane helical receptors. These receptors are coupled to heterotrimeric G-proteins comprising of $\alpha$, $\beta$ and $\gamma$ subunits which, upon activation, elicit alterations in cell calcium, initiate phosphoinositide hydrolysis or promotion or repression of cyclic adenosine monophosphate synthesis (Strader C. D. et al., 1994, *Ann. Rev. Biochem.* **63**:101-132).

Of the five pharmacologically distinct prostanoid receptors for $PGE_2$, $PGI_2$, $PGD_2$, $PGF_{2\alpha}$ and $TxA_2$ and their many isoforms, the receptor for $PGF_{2\alpha}$, also called FP receptor, shows limited tissue distribution, predominantly expressed in corpora leutea, uterine myome-

WHAT IS CLAIMED IS:

1. A G protein-coupled receptor agonist or antagonist which specifically binds to the juxtamembrane extracellular structural elements of the G protein-coupled receptor in a manner different from that of the natural ligand, and wherein said agonist or antagonist alters the transduction of an intracellular signal.

2. The agonist or antagonist of claim 1, wherein said agonist or antagonist does not crossreact with other prostanoid receptors and wherein said antagonist is effective in the presence of excess ligand.

3. The agonist or antagonist of claim 1, which comprises an amino acid sequence derived from derived from the first and/or second extracellular loops of prostanoid receptors.

4. The agonist or antagonist of claim 3, which comprises an amino acid sequence derived from the first and second extracellular loop of prostanoid receptors.

5. The antagonist of claim 1, wherein the receptor is prostaglandin $F_{2\alpha}$ receptor (FP receptor).

6. The antagonist of claim 2, which comprises amino acid sequence of the FP receptor selected from the group consisting of ILGHRDYK (PCP-8; SEQ ID NO:1); WEDRFYLL (PCP-10; SEQ ID NO:2); YQDRFYLL (PCP-14; SEQ ID NO:3); ILAHRDYK (PCP-13.7; SEQ ID NO:4); ILGFRDYK (PCP-13.11; SEQ ID NO:5); ILGHKDYK (PCP-13.13; SEQ ID NO:6); ILGHRNYK (PCP-13.14; SEQ ID NO:7); ILGHQDYK (PCP-13.18; SEQ ID NO:8); ILGHRDY-amide (PCP-13.20; SEQ ID NO:9); ILGHRDYK-amide (PCP-13.21; SEQ ID NO:1);

ILGWRDYK (PCP-13.22; SEQ ID NO:10); ILGXRDYK (PCP-13.24; SEQ ID NO:11); SNVLCSIF (PCP-15; SEQ ID NO:12); protein fusions and peptidomimetics thereof; wherein said amino acid sequence contains L- and/or D-amino acid.

7. A peptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1 to 12 and wherein said amino acid sequence contains L- and/or D-amino acid, an amino acid sequence with at least about 90% homology to SEQ ID NO:1 to 12, and peptidomimetic thereof.

8. A method for preventing premature delivery of fetus, which comprises the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist of any one of claims 1 to 6 or functional derivatives thereof.

9. A method for preventing and/or treating dysmenorrhea comprising the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist of any one of claims 1 to 6 or functional derivatives thereof.

10. A pharmaceutical composition containing at least a G protein-coupled receptor agonist of any one of claims 1 to 4, an antagonist of any one of claims 1 to 6, mixture thereof, or functional derivatives thereof in association with a pharmaceutically acceptable carrier.

11. A method of identifying a compound as a G protein-coupled receptor agonist or antagonist capable of binding to the extracellular elements of the said receptor in a manner different from that of the natural ligand, comprising the steps of:

a) culturing cells which express said receptor or identifying animal tissues *ex vivo* or *in vivo* where physiological consequences are dependent on said receptor;

b) contacting said cells or tissues with said compound to be tested for agonist or antagonist activity at said receptor; and

d) measuring a response to alter the transduction of a signal resulting in physiological consequences selected from the group consisting of increments in cell calcium, phosphoinositide hydrolysis, increased/decreased cellular cyclic adenosine monophosphate, cell growth and/or differentiation, altered gene expression, and smooth muscle contraction or dilation, wherein said response is indicative of agonist or antagonist activity.

12. A method of identifying a compound as a prostaglandin $F_2$ alpha receptor agonist or antagonist capable of binding to the extracellular elements of the said receptor in a manner different from that of the natural ligand, comprising the steps of:

a) culturing cells which express said receptor or identifying animal tissues *ex vivo* or *in vivo* where physiological consequences are dependent on said receptor;

b) contacting said cells or tissues with said compound to be tested for agonist or antagonist activity at said receptor; and

c) measuring a response to alter the transduction of a signal resulting in physiological consequences selected from the group consisting of increments in cell calcium, phosphoinositide hydrolysis, cell growth and/or differentiation, altered gene expression, and smooth muscle contraction or dilation, wherein said response is indicative of agonist or antagonist activity.

# PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

## PCT

**NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES**

(PCT Rule 47.1(c), first sentence)

To:
CÔTÉ, France
Swabey Ogilvy Renault
Suite 1600
1981 McGill College Avenue
Montréal, Québec H3A 2Y3
CANADA



| | |
|---|---|
| **Date of mailing** (day/month/year)<br>30 March 2000 (30.03.00) | |
| **Applicant's or agent's file reference**<br>12667-16PCT | **IMPORTANT NOTICE** |
| **International application No.**<br>PCT/CA99/00844 | **International filing date** (day/month/year)<br>15 September 1999 (15.09.99) |
| **Priority date** (day/month/year)<br>17 September 1998 (17.09.98) | |
| **Applicant**<br>HOPITAL SAINTE-JUSTINE et al | |

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,CN,JP,KP,KR,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,GE,
   GH,GM,HR,HU,ID,IL,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NO,NZ,OA,
   PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   30 March 2000 (30.03.00) under No. WO 00/17348

### REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

### REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

**The International Bureau of WIPO**
**34, chemin des Colombettes**
**1211 Geneva 20, Switzerland**

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

Continuation of Form PCT/IB/308

# NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

| Date of mailing (day/month/year) | IMPORTANT NOTICE |
|---|---|
| 30 March 2000 (30.03.00) | |
| **Applicant's or agent's file reference** | **International application No.** |
| 12667-16PCT | PCT/CA99/00844 |

The applicant is hereby notified that, at the time of establishment of this Notice, the time limit under Rule 46.1 for making amendments under Article 19 has not yet expired and the International Bureau had received neither such amendments nor a declaration that the applicant does not wish to make amendments.